The localization of key Bacillus subtilis penicillin binding proteins during cell growth is determined by substrate availability

Lages, Marta Carolina Afonso; Beilharz, Katrin; Angeles, Danae Morales; Veening, Jan-Willem; Scheffers, Dirk-Jan

Published in:
Environmental Microbiology

DOI:
10.1111/1462-2920.12206

Publication date:
2013

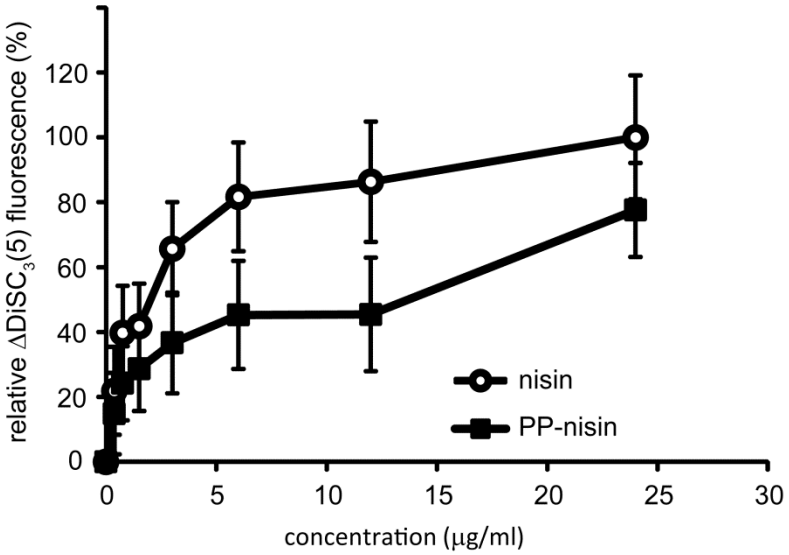


Figure S1. Lages et al.

Fig. S1. Fluorimetric measurement of the collapse of membrane potential by nisin and PP-nisin. The ΔΨ-sensitive fluorescent dye $DiSC_3(5)$ accumulates on polarized membranes of glucose energized *B. subitilis* cells, which results in fluorescence quenching. Dissipation of ΔΨ by nisin or PP-nisin is measured as release of the dye to the medium resulting in an increase in fluorescence. Various concentrations of nisin and PP-nisin were tested; values represent the mean and standard deviation from three different experiments that were performed in duplicate. Addition of nisin or PP-nisin at concentrations used to delocalize LipidII (1.5 μg ml^{-1}) resulted in a significant increase in $DiSC_3(5)$ fluorescence, indicative of a (partial) collapse of the membrane potential.

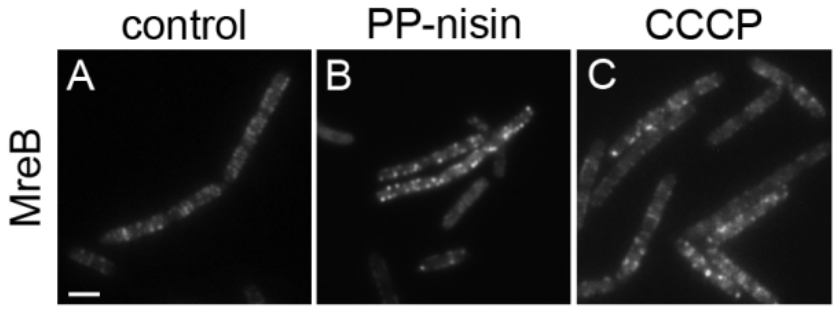


Figure S2. Lages et al.

Fig. S2. Delocalization of MreB by PP-nisin and CCCP. Localization of GFP-MreB in untreated cells (A) or after treatment with PP-nisin (B) or CCCP (C). Scale bar, same for all, 2 μm.

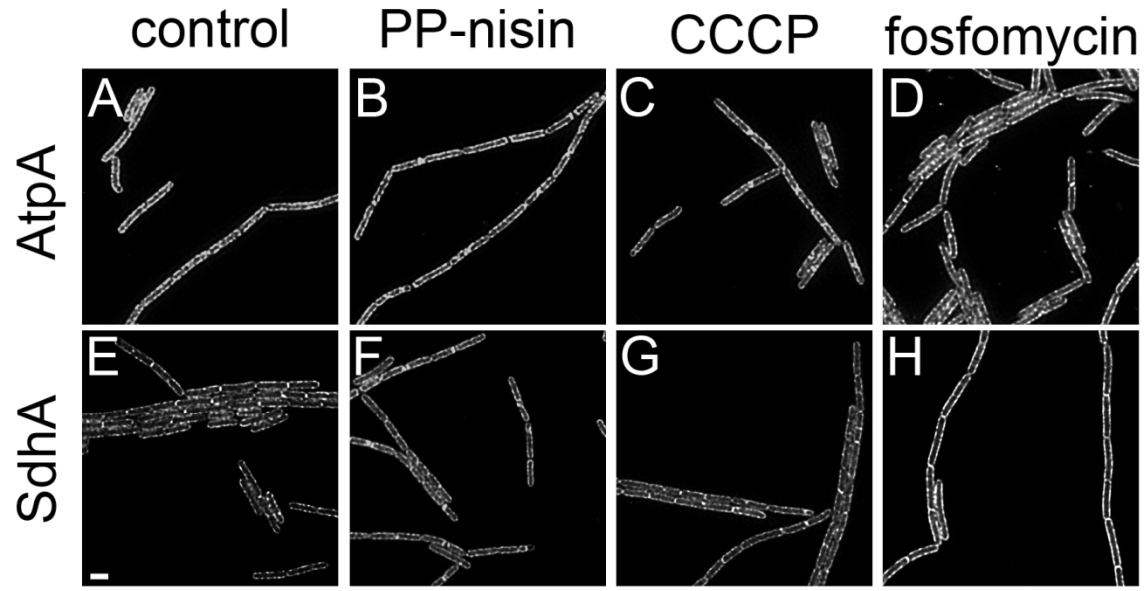
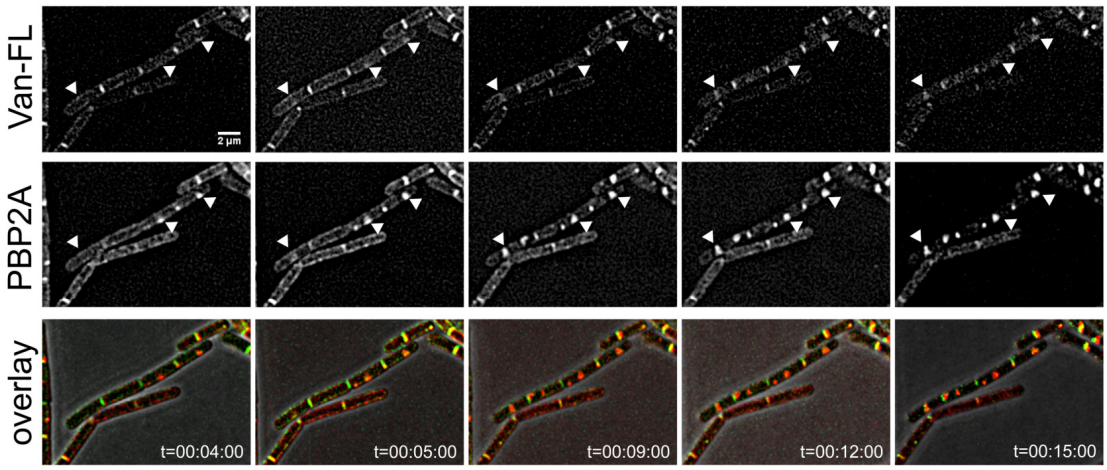


Fig. S3. Lages et al.

Fig. S3. Localization of AtpA-GFP and SdhA-GFP is not affected by PP-nisin or fosfomycin. Localization of AtpA-GFP (A-D) and SdhA-GFP (E-H) was performed in untreated cells (A,E) or after treatment with PP-nisin (B,F), CCCP (C,G), or fosfomycin (D,H). Scale bar, same for all, 2 μm. To show clear membrane localization, background light was subtracted, and out-of-focus light was removed by two-dimensional-blind deconvolution as described (Johnson *et al*., 2004).



Supplemental Figure 4. Lages et al.

Fig. S4. Time-lapse microscopy showing PG synthesis visualized by vancomycin-labelling (Van-FL, green in overlay) and localization of RFP-PBP2A (PBP2a, red in overlay) after treatment with PP-nisin. Time indicated in frames is minutes after the addition of PP-nisin. The images have been deconvolved. Scale bar 2 µm.

Movie S1. A montage of frames from the time-lapse microscopy images shown in Fig. S4.